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PATENT



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Porter, et al.

Serial No.: 09/450,999

Group Art Unit: 1624

Filing Date: November 29, 1999

Examiner: T.C. McKenzie

For: β -Alanine Derivatives

DATE OF DEPOSIT: June 19, 2002

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Jane E. Inglesse
TYPED NAME: Jane E. Inglesse
REGISTRATION NO.: 48,444

Assistant Commissioner for Patents
Washington DC 20231

TRANSMITTAL OF APPEAL BRIEF

1. Transmitted herewith in triplicate is the APPEAL BRIEF in this application with respect to the Notice of Appeal filed on April 22, 2002.
2. STATUS OF APPLICANT

- ☐ Applicant(s) has previously claimed small entity status under 37 C.F.R. §1.27.
- ☐ Applicant(s) by its/their undersigned attorney, claims small entity status under 37 C.F.R. §1.27 as:
- ☐ an Independent Inventor
 - ☐ a Small Business Concern
 - ☐ a Nonprofit Organization.

3. **EXTENSION OF TERM**

The proceedings herein are for a patent application and the provisions of 37 CFR 1.136 apply.

- ☐ Applicant petitions for an extension of time under 37 CFR 1.136 (fees: 37 CFR 1.17(a)-(d)) for the total number of months checked below:

	SMALL ENTITY		NOT SMALL ENTITY	
<input type="checkbox"/> ONE MONTH EXTENSION OF TIME	\$55	\$	\$110	\$
<input type="checkbox"/> TWO MONTH EXTENSION OF TIME	\$200	\$	\$400	\$
<input type="checkbox"/> THREE MONTH EXTENSION OF TIME	\$460	\$	\$920	\$
<input type="checkbox"/> FOUR MONTH EXTENSION OF TIME	\$720	\$	\$1440	\$
<input type="checkbox"/> FIVE MONTH EXTENSION OF TIME	\$980	\$	\$1960	\$
<input type="checkbox"/> LESS ANY EXTENSION FEE ALREADY PAID	minus	(\$)	minus	(\$)
<input checked="" type="checkbox"/> APPEAL BRIEF	\$160	\$	\$320	\$320
TOTAL FEE DUE				\$320

4. **FEE PAYMENT**

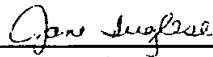
- ☒ A check in the amount of \$ 320.00 is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.
- ☐ Please charge my Deposit Account No. 23-3050 in the amount of \$____. A duplicate of this transmittal is attached.

5. **FEE DEFICIENCY**

- ☒ If any additional extension and/or fee is required, this is a request therefor and to charge Deposit Account No. 23-3050.
- ☒ If any additional fee for claims is required, charge Deposit Account No. 23-3050.

6. ☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to deposit account 23-3050. This sheet is provided in duplicate.

Date: June 19, 2002



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DOCKET NO.: CELL-0086



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John Robert Porter, John Clifford Head, Graham John Warrellow, and Sarah Catherine Archibald

Serial No.: 09/450,999

Group Art Unit: 1624

Filing Date: November 29, 1999

Examiner: Thomas C. McKenzie

For: β -ALANINE DERIVATIVES

DATE OF DEPOSIT: June 19, 2002

I HEREBY CERTIFY THAT THIS PAPER IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL, POSTAGE PREPAID ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, DC 20231.

Jane E. Inglese
JANE E. INGLESE
REGISTRATION NO.: 48,444

Assistant Commissioner for Patents
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APPELLANT'S BRIEF PURSUANT TO 37 CFR § 1.192

Appellants appeal the Final Rejection issued November 15, 2001 in connection with the above-identified patent application.

I. Real Party in Interest

Based on information supplied and to the best of the undersigned's knowledge, the real party in interest in the above-identified patent application is Celltech R&D Limited, a corporation of Great Britain, the assignee.

II. Related Appeals and Interferences

The undersigned and the assignee know of no other appeals or interferences that will directly affect, be directly affected by, or have a bearing on, the Board's decision in the pending Appeal.

III. Status of Claims

Claims 2 to 17 and 19 to 22 are pending in the present application and appear in Appendix A. Claims 1 and 18 have been cancelled. Claim 14 has been deemed allowable, and claims 2 to 13, 15 to 17, and 19 to 22 stand rejected. Appellants appeal the rejection of claims 2 to 13, 15 to 17, and 19 to 22.

IV. Status of the Amendments

The amendments filed February 15, 2002 have been entered.

V. Summary of the Invention

The physical interaction of inflammatory leukocytes with other cells is mediated by cell adhesion molecules, which have been classified according to their structure. (See specification at page 1, lines 4 to 14.) One group of adhesion molecules, named integrins, are believed to play a particularly important role in regulating immune and inflammatory responses. (See specification at page 1, lines 14 to 16.)

Integrins are cell-surface glycoproteins that form heterodimers of non-covalently linked α and β chains. (See specification at page 1, lines 14 to 16.) At least fourteen different integrin

α chains and eight different integrin β chains have been identified. (See specification at page 1, lines 17 to 19.) Integrins possessing $\alpha 4$ chains are predominantly expressed in leukocytes; consequently, their inhibition is expected to be beneficial in treating immune or inflammatory disease states. (See specification at page 3, lines 17 to 20.) The $\alpha 4$ chain can pair with either the $\beta 1$ or $\beta 7$ chain. (See specification at page 2, lines 16 to 20.)

Appellants have identified a group of β -alanine derivatives that are potent and selective inhibitors of $\alpha 4$ integrins. (See specification at page 3, lines 25 to 26.) Appellants' compounds inhibit the activity of $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins at concentrations at which they have little or no inhibitory effect against other α integrins. (See specification at page 3, lines 26 to 29.) Appellants' compounds are thus useful for the prophylaxis and treatment of diseases and disorders involving inflammation in which the extravasation of leukocytes plays a role. (See specification at page 22, lines 1 to 6.)

VI. Issues

One issue remains for resolution in this appeal:

whether the Examiner has demonstrated that those skilled in the art would find claims 2 to 13, 15 to 17, and 19 to 22 to be indefinite.

VII. Grouping of the Claims

Claims 2 to 13, 15 to 17, and 19 to 22 stand or fall together.

VIII. Argument**The Rejection under 35 U.S.C. § 112, Second Paragraph, Is Improper**

Claims 2 to 13, 15 to 17, and 19 to 22 clearly and definitely set forth the claimed subject matter. Upon review of the claims, those of ordinary skill in the art could readily determine whether a compound of interest falls within the scope of the claims.

“The test for definiteness is whether one skilled in the art would understand the bounds of the claim when read in light of the specification. If the claims read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 [second paragraph] demands no more.” *Miles Laboratories, Inc. v. Shandon Inc.*, 997 F.2d 870, 875 (Fed. Cir. 1993). If a skilled artisan can determine whether a particular chemical compound is or is not within the scope of a claim, the requirement of § 112, second paragraph has been fulfilled. *In re Miller*, 441 F.2d 689, 692 (C.C.P.A. 1971). Even a claim that reads on vast numbers of chemical compounds is not rendered indefinite thereby, so long as the boundaries of patent protection sought are clearly set forth. *In re Barr*, 444 F.2d 588, (595 C.C.P.A. 1971). *See also In re Robins*, 429 F.2d 452, 458 (C.C.P.A. 1970)(“[T]he breadth of the claims insofar as the catalyst is concerned is indeed immense. However, ‘Breadth is not indefiniteness.’”(citations omitted)).

A fundamental principle of 35 U.S.C. § 112, second paragraph is that patent Applicants are entitled to be their own lexicographers and may define the claims in whatever terms they so choose. *Fromson v. Advance Offset Plate, Inc.*, 720 F.2d 1565, 1569 (Fed. Cir. 1983); M.P.E.P. § 2173.01. Accordingly, “[t]he examiner’s focus during examination of claims for compliance with the requirement for definiteness of 35 U.S.C. 112, second paragraph is whether the claim

meets the threshold requirements of clarity and precision, *not whether more suitable language or modes of expression are available.*" *In re Swinehart*, 439 F.2d 210 (C.C.P.A. 1971); M.P.E.P. § 2173.02 (emphasis added).

The Examiner has not come forward with any credible evidence or reasoning demonstrating that those of ordinary skill in the art could not determine whether a particular compound is or is not within the scope of the claims. The Examiner asserts that the terms "heteroaliphatic group," "cycloaliphatic group," "polycycloaliphatic group," and "heteropolycycloaliphatic group" "are not recognized in the art of organic chemistry and are oxymorons. Thus, we do not know what Applicants intend by these unique terms since the prefixes conflict with the stem word aliphatic." (Office Action dated November 15, 2001, page 5). As discussed more fully below, the cited terms *are* recognized in the art of organic chemistry and those of ordinary skill in the art, therefore, understand their meaning. Thus, even if the terms used by Appellants are "oxymorons" (which Appellants do not concede), this fact alone would not be sufficient to support the present rejection.

Definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure, the teachings of the prior art, and the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. *In re Moore*, 439 F.2d 1232, 1235 (C.C.P.A. 1971); M.P.E.P. § 2173.02. When the present claim language is so examined, it is apparent that the meaning of the cited terms would be readily understood by those of ordinary skill in the art. For example, examination of the instant disclosure reveals that an explanation and description of the groups encompassed by the terms "heteroaliphatic group," "cycloaliphatic group,"

“polycycloaliphatic group,” and “heteropolycycloaliphatic group” are provided. (See, for example, page 10, lines 1 to 25 and page 11, line 5 to page 12, line 2 of the specification as filed.).¹ Moreover, numerous examples of groups encompassed by each term are listed in the specification. *Id.* Appellants are therefore at a loss to understand how the Office Action can reasonably suggest that one of ordinary skill in the art would fail to appreciate the intended meaning of the cited terms.

Furthermore, a quick search of the Internet revealed that the terms “cycloaliphatic” and “heteroaliphatic,” for example, are not only familiar to those of ordinary skill in the art, but are used in the art in a manner consistent with their use in the present application. (See attached Appendices B and C.) *The cited terms, therefore, are described in the specification and are recognized and used in the art of organic chemistry.* Those of ordinary skill in the art would understand which groups are encompassed by the terms, and would not have any difficulty determining whether a particular compound falls within the scope of the claims. The requirements of the second paragraph of 35 U.S.C. § 112 have therefore been met.

The Examiner has cited *In re Margaroli*, 318 F.2d 348 (C.C.P.A. 1963), *In re Wright*, 256 F.2d 583 (C.C.P.A. 1958), and *In re Launder*, 212 F.2d 603 (C.C.P.A. 1954) for the proposition

¹The specification states the following: “an aliphatic group...may be an optionally substituted C₁₋₁₀ aliphatic group. Particular examples include optionally substituted straight or branched chain C₁₋₆ alkyl, C₂₋₆ alkenyl, or C₂₋₆ alkynyl groups...Heteroaliphatic groups...include the aliphatic groups just described but with each group additionally containing one, two, three or four heteroatoms or heteroatom-containing groups...Optionally substituted cycloaliphatic groups...include optionally substituted C₃₋₁₀ cycloaliphatic groups. Particular examples include optionally substituted C₃₋₁₀ cycloalkyl, e.g. C₃₋₇ cycloalkyl or C₃₋₁₀ cycloalkenyl, e.g. C₃₋₇ cycloalkenyl groups...Optionally substituted heterocycloaliphatic groups...include optionally substituted C₃₋₁₀ heterocycloaliphatic groups. Particular examples include optionally substituted C₃₋₁₀ heterocycloalkyl, e.g. C₃₋₇ heterocycloalkyl, or C₃₋₁₀ heterocycloalkenyl, e.g. C₃₋₇ heterocycloalkenyl groups, each of said groups containing one, two, three or four heteroatoms or heteroatom-containing groups...Optionally substituted polycycloaliphatic groups...include optionally substituted C₇₋₁₀ bi- or tricycloalkyl or C₇₋₁₀ bi- or tricycloalkenyl groups. Optionally substituted heteropolycycloaliphatic groups...include the optionally substituted polycycloalkyl groups just described, but with each group additionally containing one, two, three or four...[hetero]atoms or groups.” Page 10, lines 1 to 13 and page 11, lines 5 to 23 (emphasis added).

that arguing that similar claims have been allowed in the past is not a valid basis for urging the allowance of pending claims, and has stated that “[t]he Examiner assumes that principle applies to non-patent literature usage of the terms.” (Advisory Action issued March 21, 2002, page 2).

Appellants respectfully submit that the cited opinions are inapposite to the present rejection because they address assertions made by Applicants that rejected claims should be allowed because similar claims present in their applications were allowed. Appellants have made no such assertion with regard to the presently rejected claims.

As best understood, the Examiner appears to be asserting that it is immaterial to a determination of the definiteness of the present claims whether the terms “cycloaliphatic” and “heteroaliphatic” have been used by others in the literature. Appellants respectfully disagree in light of the controlling legal authority discussed above. *Miles Laboratories, Inc. v. Shandon Inc.*, 997 F.2d 870, 875 (Fed. Cir. 1993); *In re Miller*, 441 F.2d 689, 692 (C.C.P.A. 1971). An indication of whether those of ordinary skill in the art understand the meaning of the cited terms is whether the terms are used in the art. Evidence that the cited terms are known and used in the art is therefore material to a proper determination of definiteness under 35 U.S.C. § 112, second paragraph, and indicates that the terms are understood by those of ordinary skill.

The Examiner has also asserted that “the heart of the indefiniteness question is if the terms are precise.” (Advisory Action issued March 21, 2002, page 2). Appellants respectfully disagree. A proper definiteness analysis focuses on whether the language used in the claims sets out and circumscribes a particular area with a *reasonable degree* of precision and particularity such that those of ordinary skill would understand the scope of the claims. The heart of a definiteness determination, therefore, is whether those of ordinary skill in the pertinent art

understand the claim language. *Credle v. Bond*, 25 F.3d 1566, 1567 (Fed. Cir. 1994)(stating that “[o]ne of ordinary skill in the art reading the claim certainly would understand the nature of the language ‘swing into the liquid’ in light of the specification and drawings. Such comprehension is all that is required by section 112 2.”).

The Examiner has further asserted, as best understood, that the passage in the specification describing heteroaliphatic groups does not indicate whether such groups may contain a heteroatom (or heteroatom-containing group) attached to the terminus of an aliphatic chain or whether such groups may contain a heteroatom (or heteroatom-containing group) positioned between two carbon atoms of an aliphatic chain. (Advisory Action dated March 21, 2002, page 2). The description provided in the specification answers the Examiner’s question and states that both possibilities are intended:

[h]eteroaliphatic groups... include the aliphatic groups just described but with each group additionally containing one, two, three or four heteroatoms or heteroatom-containing groups. Particular heteroatoms or groups include atoms or groups L^4 where L^4 is as defined above for L^2 when L^2 is a linker atom or group. *Each L^4 atom or group may interrupt the aliphatic group, or may be positioned at its terminal carbon atom to connect the group to an adjoining atom or group.*

(Specification at page 10, lines 7 to 13)(emphasis added). Examples of heteroatoms and heteroatom-containing groups (L^4 groups) are also provided in the specification at page 7, lines 24 to 32 and include -O- or -S- atoms and -C(O)-, -C(O)O-, -OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -CON(R⁸)-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)-. and -N(R⁸)SO₂N(R⁸)- groups.

Finally, the Examiner has cited *Ex parte Cavallito*, 306 F.2d 505 (C.C.P.A. 1962) for the proposition that the term “aliphatic” has been “held to be too broad” and has listed additional

cases that "cite [*Ex parte Cavallito*] with approval." (Advisory Action dated March 21, 2002, page 2). The cases cited by the Examiner are inapposite to the present rejection because they address enablement rejections rather than indefiniteness rejections. The issue decided in the cited cases was whether the scope of the claims was commensurate with that of the specification. The cases, therefore, do not provide controlling legal authority relevant to the present rejection.

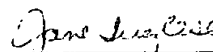
Since it is undisputed that those skilled in the art would be able to determine whether or not a compound of interest is within the scope of the claims, the rejection for alleged indefiniteness should be withdrawn.

IX. Conclusion

For the foregoing reasons, Appellants request that the present patent application be remanded to the Examiner with an instruction to both withdraw the outstanding rejection, and to allow the appealed claims.

Respectfully submitted,

Date: June 19, 2002



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APPENDIX A

2. A compound according to Claim 16 in which R is a $\text{-CO}_2\text{H}$ group.
3. A compound according to Claim 16 in which R^{a} is a hydrogen atom.
4. A compound according to Claim 16 in which R^{a} is a hydrogen atom or a hydroxyl group.
5. A compound according to Claim 16 in which $(\text{Alk}^{\text{a}})_r\text{L}^1$ is a $\text{-CON(R}^2\text{)-}$ group.
6. A compound according to Claim 5 in which $(\text{Alk}^{\text{a}})_r\text{L}^1$ is a -CONH- group.
7. A compound according to Claim 16 in which Ar^2 is a 1,4-phenylene group optionally substituted with one or two atoms or groups $\text{-L}^2(\text{Alk})_i\text{L}^3(\text{R}^4)_u$.
8. A compound according to Claim 7 in which Ar^2 is a 1,4-phenylene group.
9. A compound according to Claim 16 in which Ar^1 is a pyrimidinyl, pyridyl or phenyl group optionally substituted with one or more atoms or groups $\text{-L}^2(\text{Alk})_i\text{L}^3(\text{R}^4)_u$.

10. A compound according to Claim 9 in which Ar¹ is a pyridyl or phenyl group optionally substituted with one or more atoms or groups -L²(Alk)_iL³(R⁴)_v.
11. A compound according to Claim 10 in which Ar¹ is a 3,5-dichloropyridin-4-yl group.
12. A compound according to Claim 16 in which R¹ is the group -NHCOR³ or -NHR³.
13. A compound according to Claim 12 in which R³ is a pyrrolidinyl or thiazolidinyl group optionally substituted with one or more halogen atoms, C₁₋₆alkyl groups, haloC₁₋₆alkyl groups optionally substituted with one or more hydroxyl groups, hydroxyl groups, C₁₋₆alkoxy groups, haloC₁₋₆alkoxy groups, thiol groups, C₁₋₆alkylthio groups, aromatic groups, heteroaromatic groups, or -(Alk²)_rR¹⁰ groups, and each nitrogen atom of the pyrrolidinyl or thiazolidinyl group is optionally substituted with a group -(L⁵)_p(Alk³)_qR¹²;
or R³ is a phenyl, pyrimidinyl or 1,3,5-triazinyl group optionally substituted with one or more atoms or groups -R^{13a} or -Alk⁴(R^{13a})_m.
14. A compound which is:
3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-3-({4-[2-hydroxyethylamino]-6-methoxy-1,3,5-triazin-2-yl}amine)propanoic acid;
3-[(3,5-Dichloroisonicotinoyl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)-amino]phenyl}propanoic acid;

3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-3-[(2,6-dimethoxybenzoyl)amino]propanoic acid;

3-([(4S)-3-Acetyl-1,3-thiazolinan-4-yl]carbonyl)amino-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoic acid;

3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-3-[(2S)-1-[(3,5-dichlorophenyl)sulphonyl]tetrahydro-1-H-pyrrol-2-yl]carbonyl)amino]propanoic acid;

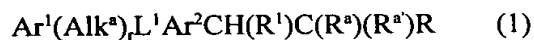
(2RS,3RS)-3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-3-[(2S)-1-[(3,5-dichlorophenyl)sulphonyl]tetrahydro-1-H-pyrrol-2-yl]carbonyl)amino}-2-hydroxypropanoic acid;

3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-3-[(2-[(2,5-dimethoxyphenyl)thio]-3-pyridinyl]carbonyl)amino]propanoic acid;

and the salts, hydrates and N-oxides thereof.

15. A pharmaceutical composition comprising a compound according to Claim 16 together with one or more pharmaceutically acceptable carriers, excipients or diluents.

16. A compound of formula (1):



wherein

Ar¹ is an aromatic or C₁₋₉ heteroaromatic group containing one to four heteroatoms selected from oxygen, nitrogen, and sulfur, and is optionally substituted with one or more atoms or groups -L²(Alk)_uL³(R⁴)_w;

L^2 and L^3 , which may be the same or different, is each a covalent bond or a divalent linker atom or group selected from -O-, -S-, -C(O)-, -C(O)O-, -OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -CON(R⁸)-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)-, and -N(R⁸)SO₂N(R⁸)-;

R⁸ is a hydrogen atom or a C₁₋₆alkyl group optionally substituted with one or more halogen atoms, hydroxy groups, or C₁₋₆alkoxy groups;

t is zero or the integer 1;

u is an integer 1, 2 or 3;

Alk is an aliphatic or heteroaliphatic chain;

R⁴ is a hydrogen or halogen atom or a group selected from C₁₋₆alkyl, -OR⁵, -SR⁵, -NR⁵R⁶, -NO₂, -CN, -CO₂R⁵, -SO₃H, -SO₃R⁵, -SOR⁵, -SO₂R⁵, -OCO₂R⁵, -CONR⁵R⁶, -OCONR⁵R⁶, -CSNR⁵R⁶, -COR⁵, -OCOR⁵, -N(R⁵)COR⁶, -N(R⁵)CSR⁶, -SO₂N(R⁵)(R⁶), -N(R⁵)SO₂R⁶, -N(R⁵)CON(R⁶)(R⁷), -N(R⁵)CSN(R⁶)(R⁷), and -N(R⁵)SO₂N(R⁶)(R⁷); and

R⁵, R⁶, and R⁷, which may be the same or different, is each a hydrogen atom or a straight or branched C₁₋₆alkyl group optionally substituted with one or more halogen atoms, hydroxy groups, or C₁₋₆alkoxy groups;

provided that when t is zero and each of L^2 and L^3 is a covalent bond, then u is the integer 1 and R⁴ is other than a hydrogen atom;

L^1 is a covalent bond or a linker atom or group selected from -CON(R²)-, -S(O)₂N(R²)-, -N(R²)-, and -O-;

R² is a hydrogen atom or a C₁₋₃ alkyl group;

Ar² is a phenylene group optionally substituted with one or two atoms or groups -L²(Alk)_iL³(R⁴)_u;

R¹ is a group selected from -NHCOR³, -NHCO₂R³, -NHR³, -NHC(O)OR³, -NHCSR³, -NHCON(R³)(R^{3a}), -NHCO₂N(R³)(R^{3a}), and -NHCSN(R³)(R^{3a});

R³ is an optionally substituted C₃₋₁₀ cycloaliphatic group, an optionally substituted C₇₋₁₀ polycycloaliphatic group, an optionally substituted C₃₋₁₀ heterocycloaliphatic group containing one, two, three or four heteroatoms or heteroatom-containing groups selected from -O-, -S-, -C(O)-, -C(O)O-, OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -C(O)NR⁸-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)- and -N(R⁸)SO₂N(R⁸)-; an optionally substituted C₇₋₁₀ heteropolycycloaliphatic group containing one, two, three or four heteroatoms or heteroatom-containing groups selected from -O-, -S-, -C(O)-, -C(O)O-, OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -C(O)NR⁸-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)- and -N(R⁸)SO₂N(R⁸)-; an optionally substituted aromatic group, or an optionally substituted C₁₋₉ heteroaromatic group containing one, two, three or four heteroatoms selected from oxygen, nitrogen, and sulfur;

R^{3a} is a hydrogen atom, an optionally substituted C₁₋₆ aliphatic group, an optionally substituted C₁₋₆ heteroaliphatic group containing one, two, three or four heteroatoms or heteroatom-containing groups selected from -O-, -S-, -C(O)-, -C(O)O-, OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -C(O)NR⁸-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)-

and -N(R⁸)SO₂N(R⁸)-, an optionally substituted C₃₋₁₀ cycloaliphatic group, an optionally substituted C₇₋₁₀ polycycloaliphatic group, an optionally substituted C₃₋₁₀ heterocycloaliphatic group containing one, two, three or four heteroatoms or heteroatom-containing groups selected from -O-, -S-, -C(O)-, -C(O)O-, OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -C(O)NR⁸-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)- and -N(R⁸)SO₂N(R⁸)-; an optionally substituted C₇₋₁₀ heteropolycycloaliphatic group containing one, two, three or four heteroatoms or heteroatom-containing groups selected from -O-, -S-, -C(O)-, -C(O)O-, OC(O)-, -C(S)-, -S(O)-, -S(O)₂-, -N(R⁸)-, -C(O)NR⁸-, -OC(O)N(R⁸)-, -CSN(R⁸)-, -N(R⁸)CO-, -N(R⁸)C(O)O-, -N(R⁸)CS-, -S(O)₂N(R⁸)-, -N(R⁸)S(O)₂-, -N(R⁸)CON(R⁸)-, -N(R⁸)CSN(R⁸)- and -N(R⁸)SO₂N(R⁸)-; an optionally substituted aromatic group, or an optionally substituted C₁₋₉ heteroaromatic group containing one, two, three or four heteroatoms selected from oxygen, nitrogen, and sulfur;

wherein the optional substituents for the aromatic groups and the heteroaromatic groups of R³ and R^{3a} are selected from one or more atoms or groups R¹³ wherein R¹³ is -R^{13a} or -Alk⁴(R^{13a})_m;

R^{13a} is a halogen atom, or an amino, substituted amino, nitro, cyano, amidino, hydroxyl, substituted hydroxyl, formyl, carboxyl, esterified carboxyl, thiol, substituted thiol, -COR¹⁴-, -CSR¹⁴-, -SO₃H-, -SOR¹⁴-, -SO₂R¹⁴-, -SO₂NH₂-, -SO₂NHR¹⁴-, -SO₂N(R¹⁴)₂-, -CONH₂-, -CSNH₂-, -CONHR¹⁴-, -CSNHR¹⁴-, -CON(R¹⁴)₂-, -CSN(R¹⁴)₂-, -N(R¹¹)SO₂R¹⁴-, -N(SO₂R¹⁴)₂-, -N(R¹¹)SO₂NH₂-, -N(R¹¹)SO₂NHR¹⁴-, -N(R¹¹)SO₂N(R¹⁴)₂-, -N(R¹¹)COR¹⁴-, -N(R¹¹)CONH₂-, -N(R¹¹)CONHR¹⁴-, -N(R¹¹)CON(R¹⁴)₂-, -N(R¹¹)CSNH₂-, -N(R¹¹)CSNHR¹⁴-, -N(R¹¹)CSN(R¹⁴)₂-,

-N(R¹¹)CSR¹⁴, -N(R¹¹)C(O)OR¹⁴, -SO₂NHet¹, -CONHet¹, -CSNHet¹, -N(R¹¹)SO₂NHet¹,
-N(R¹¹)CONHet¹, -N(R¹¹)CSNHet¹, -SO₂N(R¹¹)Het², -Het², -CON(R¹¹)Het², -CSN(R¹¹)Het²,
-N(R¹¹)CON(R¹¹)Het², -N(R¹¹)CSN(R¹¹)Het², aryl or heteroaryl group;

R¹⁴ is an -Alk⁴(R^{13a})_m, aryl or heteroaryl group;

NHet¹ is a C₅₋₇cyclicamino group optionally containing one or more -O- or -S- atoms or -N(R¹¹)-, -C(O)- or -C(S)- groups and optionally substituted with one or more substituents as defined for the cycloaliphatic groups of R³ and R^{3a};

Het² is a monocyclic C₅₋₇carbocyclic group optionally containing one or more -O- or -S- atoms or -N(R¹¹)-, -C(O) or -C(S)- groups and optionally substituted with one or more substituents as defined for the cycloaliphatic groups of R³ and R^{3a};

Alk⁴ is a straight or branched C₁₋₆alkylene, C₂₋₆alkenylene or C₂₋₆alkynylene chain, optionally interrupted by one, two, or three -O- or -S- atoms or -S(O)_n or -N(R¹⁵)- groups;

R¹⁵ is a hydrogen atom or C₁₋₆alkyl group;

m is zero or an integer 1, 2 or 3;

n is an integer 1 or 2;

wherein the optional substituents for the aliphatic groups and the heteroaliphatic groups of R^{3a} are selected from halogen atoms, hydroxy groups, C₁₋₆alkoxy groups, thiol groups, C₁₋₆alkylthio groups, amino groups, and substituted amino groups;

wherein the optional substituents for the cycloaliphatic, polycycloaliphatic, heterocycloaliphatic and heteropolycycloaliphatic groups of R³ and R^{3a} are selected from halogen atoms, C₁₋₆alkyl groups, haloC₁₋₆alkyl groups optionally substituted with hydroxyl

groups, hydroxyl groups, C₁₋₆alkoxy groups, haloC₁₋₆alkoxy groups, thiol groups, C₁₋₆alkylthio groups, aromatic groups, heteroaromatic groups, and -(Alk²)_vR¹⁰ groups;

Alk² is a straight or branched C₁₋₃ alkylene chain;

v is zero or an integer 1;

R¹⁰ is a -OH, -SH, -N(R¹¹)₂, -CN, -CO₂R¹¹, -NO₂, -CON(R¹¹)₂, -CSN(R¹¹)₂, -OC(O)N(R¹¹)₂, -C(O)H, -COR¹¹, -OCO₂R¹¹, -OC(O)R¹¹, -C(S)R¹¹, -CSN(R¹¹)₂, -N(R¹¹)COR¹¹, -N(R¹¹)CSR¹¹, -SO₃H, -SOR¹¹, -SO₂R¹¹, -SO₃R¹¹, -SO₂N(R¹¹)₂, -N(R¹¹)SO₂R¹¹, -N(R¹¹)CON(R¹¹)₂, -N(R¹¹)CSN(R¹¹)₂, or -N(R¹¹)SO₂N(R¹¹)₂ group; and

R¹¹ is an atom or group as defined for R⁸ or an optionally substituted cycloaliphatic or heterocycloaliphatic group as defined for R³;

and when R³ is a heterocycloaliphatic group containing one or more nitrogen atoms each nitrogen atom is optionally substituted with a group -(L⁵)_p(Alk³)_qR¹²;

L⁵ is -C(O)-, -C(O)O-, -C(S)-, -S(O)-, -S(O)₂-, -CON(R¹¹)-, -CSN(R¹¹)-, -SON(R¹¹)- or -SO₂N(R¹¹)-;

p is zero or an integer 1;

Alk³ is an optionally substituted aliphatic or heteroaliphatic chain;

q is zero or an integer 1;

R¹² is a hydrogen atom or an optionally substituted cycloaliphatic, heterocycloaliphatic, polycycloaliphatic, polyheterocycloaliphatic, aromatic or heteroaromatic group;

R^a and R^{a'}, which may be the same or different, are each independently selected from a hydrogen or halogen atom or an optionally substituted straight or branched

alkyl, alkenyl, alkynyl, haloalkyl, alkoxy, haloalkoxy, alkylthio or $-(\text{Alk}^b)_m\text{R}^b$ group (in which Alk^b is a C_{1-3} alkylene chain, m is zero or the integer 1, and R^b is $-\text{OH}$, $-\text{SH}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{R}^c$ (where R^c is an optionally substituted straight or branched C_{1-6} alkyl group), $-\text{SO}_3\text{H}$, $-\text{SOR}^c$, $-\text{SO}_2\text{R}^c$, $-\text{SO}_3\text{R}^c$, $-\text{OCO}_2\text{R}^c$, $-\text{C}(\text{O})\text{H}$, $-\text{C}(\text{O})\text{R}^c$, $-\text{OC}(\text{O})\text{R}^c$, $-\text{C}(\text{S})\text{R}^c$, $-\text{NR}^d\text{R}^e$ (where R^d and R^e , which may be the same or different, are each a hydrogen atom or an optionally substituted straight or branched C_{1-6} alkyl group), $-\text{CON}(\text{R}^d)(\text{R}^e)$, $-\text{OC}(\text{O})\text{N}(\text{R}^d)(\text{R}^e)$, $-\text{N}(\text{R}^d)\text{C}(\text{O})\text{R}^e$, $-\text{CSN}(\text{R}^d)(\text{R}^e)$, $-\text{N}(\text{R}^d)\text{C}(\text{S})\text{R}^e$, $-\text{S}(\text{O})_2\text{N}(\text{R}^d)(\text{R}^e)$, $-\text{N}(\text{R}^d)\text{SO}_2\text{R}^e$, $-\text{N}(\text{R}^d)\text{CON}(\text{R}^e)(\text{R}^f)$ (where R^f is a hydrogen atom or an optionally substituted straight or branched C_{1-6} alkyl group), $-\text{N}(\text{R}^d)\text{C}(\text{S})\text{N}(\text{R}^e)(\text{R}^f)$ or $-\text{N}(\text{R}^d)\text{SO}_2\text{N}(\text{R}^e)(\text{R}^f)$ group);

Alk^a is an optionally substituted C_{1-6} aliphatic or C_{1-6} heteroaliphatic chain containing one, two, three or four heteroatoms or heteroatom-containing groups selected from $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, $\text{OC}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{N}(\text{R}^8)-$, $-\text{C}(\text{O})\text{NR}^8-$, $-\text{OC}(\text{O})\text{N}(\text{R}^8)-$, $-\text{CSN}(\text{R}^8)-$, $-\text{N}(\text{R}^8)\text{CO}-$, $-\text{N}(\text{R}^8)\text{C}(\text{O})\text{O}-$, $-\text{N}(\text{R}^8)\text{CS}-$, $-\text{S}(\text{O})_2\text{N}(\text{R}^8)-$, $-\text{N}(\text{R}^8)\text{S}(\text{O})_2-$, $-\text{N}(\text{R}^8)\text{CON}(\text{R}^8)-$, $-\text{N}(\text{R}^8)\text{CSN}(\text{R}^8)-$, and $-\text{N}(\text{R}^8)\text{SO}_2\text{N}(\text{R}^8)-$;

wherein the optional substituents for the aliphatic and heteroaliphatic groups of Alk^a are selected from halogen atoms, hydroxy groups, C_{1-6} alkoxy groups, thiol groups, C_{1-6} alkylthio groups, amino groups, and substituted amino groups;

r is zero or the integer 1;

R is a carboxylic acid (CO_2H), a carboxylic ester group, or carboxylic amide group;

and the salts, hydrates and N-oxides thereof.

17. A method for the treatment of a mammal suffering from inflammatory arthritis, multiple sclerosis, allograft rejection, diabetes, inflammatory dermatoses, asthma or inflammatory bowel disease, comprising administering to the mammal a therapeutically effective amount of a compound according to Claim 16.

19. A method according to Claim 18 wherein said inflammatory arthritis is selected from the group consisting of rheumatoid arthritis vasculitis and polydermatomyositis.

20. A method according to Claim 19 wherein said inflammatory dermatoses are selected from the group consisting of psoriasis and dermatitis.

21. A method for inhibiting, in a mammal, the binding of $\alpha 4$ integrins to the ligands thereof, comprising administering to the mammal an effective amount of a compound according to Claim 16.

22. A method according to Claim 21 wherein the $\alpha 4$ integrins are selected from the group consisting of $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins.

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APPENDIX B

DuPont Nylon Intermediates and Specialties

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Display Products by Functional Group



Amines

DuPont offers a line of multifunctional aliphatic, cycloaliphatic, and aromatic amine products, which have been used in a wide variety of applications including fibers, coatings, elastomers, resins, adhesives, and scale and corrosion inhibitors. These products undergo typical amine reactions to form polyamides, isocyanates, ureas, and epoxy curing agents.

- [\(BHMT-HP\) Bis\(hexamethylene\)triamine-High Purity, 98%](#)
- [\(BHMT Amine\) Bis\(hexamethylene\)triamine](#)
- [\(DCH-99\) 1,2-Diaminocyclohexane](#)
- [\(DYTEK® EP Diamine - DAMP\) 1,3-Pentanediamine](#)
- [\(DYTEK® A Amine - MPMD\) 2-Methylpentamethylenediamine](#)
- [\(HMD\) Hexamethylenediamine, Solution](#)
- [\(HMD\) Hexamethylenediamine, Anhydrous](#)
- [\(HMI\) Hexamethyleneimine](#)

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Boron Products

DuPont has recently introduced two new boron products. Triisopropyl borate (TIPB) and Triphenylboron (TPB). Due to the diverse properties of these molecules, these products can be used in a wide variety of applications including catalyst, fuel and antifoulant additives, lubricants and precursors to boronic acids used in Suzuki coupling reactions to name a few.

- [\(TIPB\) Triisopropyl Borate](#)
- [\(TPB\) Triphenylboron](#)

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Carboxylic Acids

DuPont dicarboxylic acids exhibit typical carboxyl group chemistry leading to a variety of products serving many applications. The products can be used to formulate polyester polyols, plasticizers, chelating agents, corrosion inhibitors, and cleaning agents.

- [Adi-pure® High Purity Adipic Acid](#)
- [CORFREE® M1 Corrosion Inhibitor Raw Materials](#)
- [\(DBA\) Dibasic Acid](#)
- [\(DDDA\) Dodecanedioic Acid](#)

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Cyclics

DuPont offers a family of large-ring cycloolefinic and cycloaliphatic compounds. The cycloolefins undergo addition reactions with halogens to give products useful in flame retardants, flavors and fragrances; as monomers in polyolefin synthesis and reactants in other organic syntheses; and as solvents.

- [\(CDDA\) Cyclododecanol](#)
- [\(CDDA-HP\) Cyclododecanol - HP](#)
- [\(CDDK\) Cyclododecanone](#)
- [\(CDDT\) Cyclododecatriene](#)
- [\(COD\) Cyclooctadiene](#)
- [\(VCH\) Vinylcyclohexene](#)
- [XOLVONE™ DMPD Dimethyl-2-piperidone](#)

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Esters

DuPont's intermediates include dibasic esters of both aliphatic and aromatic carboxylic acids. These esters fulfill a variety of needs as chemical intermediates and as solvents for coatings, industrial cleaning compounds, inks, fabric dyes, and chemical reactions. They undergo reactions typical of esters, including transesterification, hydrolysis, and reduction, to yield commercially significant products.

- [\(DBEs\) Dibasic Esters](#)
- [\(DBE-IB\) Diisobutyl Esters](#)
- [DBE Microemulsion Concentrate](#)

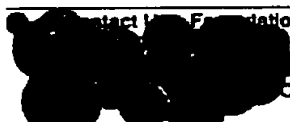
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Nitriles

DuPont high-purity nitriles are highly polar liquids that can be used as reaction or crystallization solvents or as intermediates in the manufacture of acids, amines, amides, and other products.

- [\(ADN\) Adiponitrile](#)
- [\(MGN\) 2-Methylglutaronitrile](#)
- [\(2PN-HP\) cis-2-Pentenenitrile, High Purity](#)

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UM-BBD Organic Functional Groups

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This is a list of 50 organic functional groups found in at least one UM-BBD compound, and, for each functional group, at least one UM-BBD compound which contains it. The UM-BBD contains many more examples of the most common groups. A [graphic version](#) of this list and a list of UM-BBD pathways ordered by functional group also exist.

Organic Functional Group	Representative UM-BBD Compounds
Methane	Methane
Alkane, primary	n-Octane
Alkane, secondary	p-Cymene
Alkane, tertiary	Methyl-tert-butyl ether
Cycloaliphatic ring	1-Aminocyclopropane-1-Carboxylate ; Cyclohexanol
Bicycloaliphatic ring	(+)-Camphor
Tricycloaliphatic ring	Adamantanone
Alkene	Propylene ; Styrene
Alkyne	Acetylene
Monocyclic aromatic hydrocarbon	Toluene ; Ethylbenzene
Polycyclic aromatic hydrocarbon	Naphthalene ; Phenanthrene ; Fluorene
Biphenyl-type benzenoid ring	Biphenyl ; 4-Chlorobiphenyl
Oxygen ether	Methyl-tert-butyl ether ; Tetrahydrofuran
Thioether	Dimethyl sulfide ; Methionine ; Prometryn
S-heterocyclic ring	Dibenzothiophene
N-heterocyclic ring, saturated	Atrazine ; Nicotine ; Carbazole ; 3-Methylquinoline
N-heterocyclic ring, unsaturated	Nicotine
O-heterocyclic ring	Dibenzofuran
Epoxide	Trichloroethylene epoxide ; Propylene oxide ; (RS)-3-Chloro-1,2-epoxypropane
Peroxide	Octane hydroperoxide
Ketone	Methylethylketone
Thioketone	Carbon disulfide
Alcohol	o-, m- and p-Cresol ; Orcinol ; Pentachlorophenol ; 1,3-Dichloro-2-propanol

Thiol	<u>Methanethiol</u>
Amine, primary	<u>2-Aminobenzoate</u>
Amine, secondary	<u>Glyphosate</u>
Amine, tertiary	<u>Nitrilotriacetate</u>
Aldehyde	<u>3-Hydroxybenzaldehyde</u>
Carboxylic acid	<u>3-Phenylpropionate; o-Phthalic acid</u>
Carboxylic acid ester	<u>Butyrolactone</u>
Carboxylic thioester	<u>Benzoyl-S-CoA</u>
Amide	<u>Acrylamide; Caprolactam</u>
Nitrile	<u>Acrylonitrile; Bromoxynil; Benzonitrile</u>
Oxime	<u>Z-Phenylacetaldoxime</u>
Thiocyanate	<u>Thiocyanate anion</u>
Cyanamide	<u>Cyanamide</u>
Nitro	<u>Nitrobenzene; Trinitrotoluene; 4-Nitrophenol; 2-Nitropropane</u>
Nitrate ester	<u>Pentaerythritol tetranitrate; Nitroglycerin</u>
Diazo	<u>4-Carboxy-4'-sulfoazobenzene</u>
Organohalide	<u>1,1,1-Trichloro-2,2-bis-(4'- chlorophenyl)ethane; Trichloroethylene; Methylfluoride; Tetrachlorethylene; 1,2,4-Trichlorobenzene</u>
Organomercurial	<u>Methylmercury chloride</u>
Organoarsenical	<u>Arsonoacetate</u>
Organosilicon	<u>Octamethylcyclotetrasiloxane</u>
Organotin	<u>Tri-n-butyltin</u>
Organophosphate ester	<u>Paraoxon</u>
Thiophosphate ester	<u>Parathion</u>
Phosphonic acid	<u>Glyphosate</u>
Phosphinic acid	<u>Dimethylphosphinic acid</u>
Sulfonic acid	<u>Methanesulfonic acid; p-Toluenesulfonic acid</u>
Sulfate ester	<u>Dodecyl sulfate</u>

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August 24, 2001 BBDMaster@email.labmed.umn.edu

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APPENDIX C

resulting in lower aromatic carbon and increased carboxyl and aliphatic carbon content.

APPENDIX F: PRELIMINAR DATA FOR WETLAND-HABITAT PONDS

In addition to the primary study of DOC and THM precursors released from peat soils under agricultural production, soil water from three wetland-habitat test ponds were sampled by the USGS and samples were analyzed by the DWR Bryte Laboratory for the same parameters discussed in appendix A. The test ponds were designed to evaluate the effects of different wetland habitats on land subsidence in the Delta. Microbial oxidation of peat soil is the primary cause of currently observed land subsidence in the Delta (Deverel and others, 1998). Implementation of wetland habitats is being tested to evaluate how these treatments affect subsidence compared to nonwetland land uses (for example, drained pastures, agriculture). As part of this study, three test ponds also were monitored for potential effects of the wetlands on water quality, in particular DOC and THM precursor release.

Two of the three wetland-habitat test ponds consisted of 30-ft square enclosures, and the third test pond was a small (about 100 ft²) spring-fed pond (TwitPiz5) in a subsidence mitigation test area being maintained by DWR. One of the enclosed test ponds is flooded continuously (TwitPiz6) with about 1 to 1.5 ft of water maintained above land surface. This flooded pond has been in operation for about 4 years, and results have indicated that shallow flooding decreases subsidence rates by a factor of about four and also encourages the growth of cattails that contribute biomass back to the system. The second enclosed pond is a reverse-flooding treatment pond (TwitPiz7) that is intentionally flooded to a depth of about 1 ft above land surface from early spring until mid-July. Winter precipitation kept this pond moist to very wet (for example, standing water) during the later part of December 1996 and through February 1997.

Stainless-steel piezometers were installed in each test pond to sample water from 0.5 to 1.5 ft below land surface. In August 1996, surface water in the ponds also was analyzed. Samples were collected from the open-water and continuously flooded ponds for the September and November 1996 samplings. Results of these analyses are presented in tables F1 through F4.

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Thiophilic adsorption chromatography on Fractogel® EMD TA

Advantage of the tentacle mediated thiophilic adsorption

The high efficiency of the thiophilic adsorption chromatography is based on the specific interaction of a covalently bound sulphur containing ligand immobilized on a chromatographic support with its counterpart. The chromatographic support is designed especially for the efficient isolation of antibodies.

One disadvantage of conventional affinity chromatographic media is their relatively low protein binding capacity. In order to improve this, the amount of bound ligand should be increased. The new Fractogel® EMD TA for thiophilic adsorption chromatography is synthesized according to the tentacle technology where the group specific ligands are present in a high density. Thus, the thiophilic tentacle material has a high protein binding capacity and is suitable to purify antibodies in an analytical as well as in a preparative scale.

However, more than the density the steric accessibility of the ligand is related to a suitable capacity of an affinity gel. As known from the tentacle type ion exchangers the linear polymer chains provide an appropriate spacing which results in minimized non specific interactions with proteins combined with high protein binding capacities. In addition, the special chemistry of the 3-S type ligand that is present in Fractogel® EMD TA allows significantly better thiophilic bindings whereas the 2-S types have also hydrophobic features.

Application area

The main application area of the specific chromatographic support carrying a sulphur containing ligand is the isolation of proteins with thiophilic regions and peptides with aromatic amino residues. The corresponding technique is called thiophilic adsorption chromatography. This chromatographic method is based on a salt promoted adsorption of proteins to a sulfone and thioether containing heteroaliphatic ligand. The binding of the protein takes place mainly via accessible tryptophane and/or phenylalanine residues. Corresponding motifs can be observed within conserved regions of various antibodies.

Therefore thiophilic adsorption chromatography is very useful for the purification of immunoglobulins (monoclonal and polyclonal antibodies). Albumins are not adsorbed on thiophilic media, which often simplifies the effective separation of antibodies

Antibodies of the IgM sub-class can also be bound on a Fractogel® EMD TA column. The binding of antibodies from different species to thiophilic adsorption supports offers advantages compared to the protein A method. All antibodies tested so far bind to Fractogel® EMD TA, as shown in the table. Due to the gentle elution conditions at physiological pH values high recoveries of biological active antibodies can be obtained.

Certain other proteins and peptides carrying thiophilic areas located on their surface can also be isolated by this technique, using Fractogel® EMD TA. Operating at high concentrations of ammonium sulphate, Fractogel® EMD TA creates hydrophobic properties providing special selectivities for hydrophobic proteins.

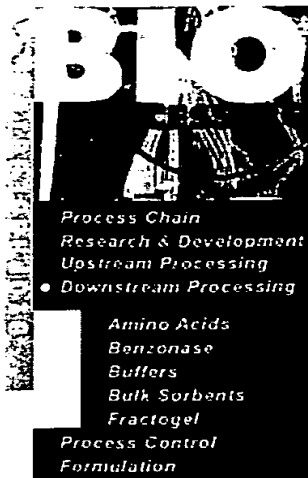
Regeneration

Short term treatment with sodium hydroxide solution (0.1 to 0.5 M) is best suited for the regeneration of Fractogel® EMD TA. Regeneration can also be performed by rinsing with 50 % ethylene glycol. Another successful method to remove tightly bound or denaturated material from the column is to rinse with 20 % ethanol or 6 M urea. For regeneration of Fractogel® media with organic solvents a linear flow rate of 1 cm/min should not be exceeded.

Antibody-binding characteristics of Fractogel® EMD TA

Species	Fractogel® EMD TA
Horse IgG	+
Rat IgG	+
Goat IgG	+
Chicken IgG	+
Bovine IgG	+
Rabbit IgG	+
Sheep IgG	+
Dog IgG	+
Pig IgG	+
Cat IgG	+
Mouse IgG	+
Recombinant ab (scFv)	+
Human IgG3	+
Chicken (yolk) IgY	+
Human IgM	+

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LABORATORY->BIOPROCESSING->DOWNSTREAM->Fractogel

Fractogel® EMD process media Introduction to Thiophilic Adsorption Chromatography (TAC)

Fractogel® EMD TA is an affinity resin designed specifically for the purification of antibodies and can be utilised instead of ion exchangers or in combination with other methods. The high efficiency of the thiophilic adsorption chromatography is based on the specific interaction of a covalently bound sulphur containing ligand immobilized on a chromatographic support with its counterpart. The mechanism is based on a salt promoted adsorption of proteins to a sulfone and thioether containing heteroaliphatic ligand. The binding of the protein takes place mainly via accessible tryptophane and/or phenylalanine residues. Corresponding motifs can be observed within conserved regions of various antibodies. Unlike Protein A, antibodies can be eluted at physiological pH conditions. Albumins are not adsorbed on thiophilic media, which often simplifies the effective separation of antibodies.

Antibodies of the IgM sub-class and recombinant antibodies produced in *E. coli* can also be purified.

Getting started now (Practical hints)

The best results for antibody isolations are obtained at ammonium sulphate concentrations between 0.8 M and 1.5 M. The elution can be achieved applying a decreasing gradient of the concentration of ammonium sulphate. Although in affinity chromatography often a step wise gradient for elution is preferred, sometimes gently decreasing salt gradients can provide better separations.

Metal Chelate Affinity Chromatography	Ion Exchange Chromatography	Hydrophobic Interaction Chromatography	Molecularsize Exclusion Chromatography
Activated resin	Application areas	Regeneration	Benefits
		Technical info	Properties
Fractogel® EMD	Product range	Data Sheets	FAQ



Partial List of Abstracts, 1992

Aiken, G. R., 1992, Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method: Environmental Science and Technology, v. 26, p. 2435-2439.

One of the common methods for determining DOC concentrations in water samples used wet oxidation with persulphate. The effects of chloride ions on the determination of DOC by this method were investigated in a gas-tight reaction chamber at a temperature of 100°C for 5 minutes. The presence of chloride in concentrations greater than 0.02 M interfered with the analysis of aqueous DOC concentrations by the wet oxidation method when a reaction time of 5 minutes was used. Chloride competed with DOC for persulphate, lowering the overall oxidation efficiency. The oxidation of chloride produced hypochlorous acid which reacted with DOC to produce chlorinated intermediate compounds. The interference could be removed by increasing the reaction time or by diluting samples so that the chloride concentration was less than 0.02 M. A method that used persulphate oxidation combined with UV irradiation was unaffected by the presence of chloride.

Aiken, G.R., McKnight, D.M., Thorn, K.A., and Thurman, E.M., 1992, Isolation of hydrophilic organic acids from water using nonionic macroporous resins: Organic Geochemistry, v. 18, no. 4, p. 567-573.

A method was developed for the isolation of hydrophilic organic acids from aquatic environments using Amberlite XAD-4 resin. The method used a 2 column array of XAD-8 and XAD-4 resins in series. The hydrophobic organic acids, composed primarily of aquatic fulvic acid, were removed from the sample on XAD-8, followed by the isolation of the more hydrophilic organic acids on XAD-4. For samples from several diverse environments, more of the dissolved organic carbon was isolated on the XAD-8 resin (23-58 per cent) than on the XAD-4 resin (7-25 per cent). For these samples, the hydrophilic acids have lower carbon and hydrogen contents, higher oxygen and nitrogen contents, and are lower in molecular weight than the corresponding fulvic acids. Carbon-13 NMR analyses indicated that the hydrophilic acids have a lower concentration of aromatic carbon and greater heteroaliphatic, ketone and carboxyl content than the fulvic acids.

Alpers, C.N., Nordstrom, D.K., and Burchard, J.M., 1992, Compilation and interpretation of water quality and discharge data for acid mine waters at Iron Mountain, Shasta County, California, 1940-91: U.S. Geological Survey Water-Resources Report, 91-4160, 173 p.

This report contains a compilation and interpretation of the historical records of water quality and discharge for the period 1940-91 from the two most significant discharge points for acid mine drainage at Iron Mountain, Shasta County, California--the Richmond and Lawson portals. The primary objective is to formulate a conceptual model of subsurface processes that accounts for trends with time of water quality and discharge volume from the two mine portals. It is proposed that Zn/Cu ratios in the effluent waters are controlled by alternating periods of precipitation and dissolution of Fe-sulfate minerals such as melanterite ((Fe(II), Zn, Cu) SO₄*7H₂O)). Copper is concentrated



Dissolved Organic Carbon Concentrations and Compositions, and Trihalomethane Formation Potentials in Waters from Agricultural Peat Soils, Sacramento-San Joaquin Delta, California: Implications for Drinking-Water Quality

By Roger Fujii, Anthony J. Ranalli, George R. Aiken, and Brian A. Bergamaschi

U.S. Geological Survey
Water-Resources Investigations Report 98-4147

APPENDICIES

Appendix A: Field and Sample Collection Methods

Appendix B: Dissolved Organic Carbon and Ultraviolet Absorption Measurements

Appendix C: Dissolved Organic Carbon Fractionation and Isolation

Appendix D: U.S. Geological Survey Trihalomethane Methods

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APPENDIX A: FIELD AND SAMPLE COLLECTION METHODS

Installation of Samplers

Four sampling sites were established along an east-west transect at the northern end of the agricultural field (fig. 2). These sites were spaced about 40 ft apart and about 50 ft south of the ditch that drains the field. The sites were located to sample subsurface drainage from the field. At each site, lysimeters (1.5-in. diameter, 6-in. long, with 0.2-mm pores) were installed to sample soil water from 0.5 to 1.5 ft below land surface, and piezometers (1.25-in. diameter, 2 ft long, with 0.02-in. slots) were installed to sample ground water from 4.5 to 6.5 ft below land surface (fig. 3). The lysimeters, piezometers, and extension tubes were constructed of 316L stainless steel to prevent carbon contamination from sampling materials.

The holes for the installation of the lysimeters and the piezometers were drilled using a gas-powered, tower-mounted drill with a 4-in. bit. The holes were evacuated using a 1-in. water pump. The piezometers were lowered onto a bed of sand (#2/12), and the annular space was packed with sand to about 1 ft above the sampling interval. The remaining annular space was filled with bentonite grout to land surface. The lysimeters were placed on a bed of silica flour, a silica flour slurry was packed around the sampler, and bentonite grout was used to seal the hole from the top of the sampler to land surface. After installation, the piezometers initially were developed using a peristaltic pump until the effluent was clear. Lysimeters initially were developed after the field was flooded.

Sample Collection and Field Measurements

Lysimeters are designed to sample soil water from unsaturated soils, which, in contrast to piezometer sampling of ground water, usually do not yield large sample volumes. Thus, development of lysimeters prior to sampling involved evacuating or purging the lysimeter using a peristaltic pump and either Teflon or c-flex tubing and stainless-steel tubing. These sampling materials were used to

prevent carbon contamination of samples. After initial purging, the lysimeters were evacuated every 15 to 20 minutes, and the soil water samples were collected in 1-gal, baked, amber glass bottles placed in a bucket of ice to keep the samples chilled and to slow microbial decomposition of DOC. Because of the small volume of soil water available under unsaturated soil conditions, lysimeter sampling continued throughout the day to yield the maximum amount of sample possible.

Temperature of the lysimeter samples was measured immediately on the purged soil water. A 15- to 20-mL subsample from the lysimeter sample was measured for pH and specific conductance in the field. The pH and specific conductance meters and probes were calibrated, checked, and recalibrated, if necessary, before each measurement. The pH values for the lysimeter samples are questionable because of degassing of CO₂ and exposure to atmospheric gases during sampling.

Samples were pressure filtered (0.45 µm) in the field using a peristaltic pump. The pump, tubing, and filter were flushed with at least 1 L of deionized water and 75 to 100 mL of sample water prior to collecting the samples. Analysis of organic-free water filtered through the set up after the deionized water rinse yielded a DOC concentration of < 0.2 mg/L, indicating no contamination of samples from the filtering procedure.

Piezometers also were sampled using a peristaltic pump and Teflon or c-flex tubing and stainless-steel tubing. Prior to sample collection, at least three casing volumes (about 2.5 gal) of water were purged, and then 20-mL test samples were collected every 5 to 10 minutes to test piezometer development. Specific conductance of the 20-mL test samples was measured, and samples for laboratory analysis were collected only after successive specific-conductance measurements were within 5 percent of each other, indicating that aquifer water was being pumped. Sample water was collected in 1-gal, baked, amber glass bottles, and temperature and pH were immediately measured for a 20-mL subsample. Piezometer samples were pressure filtered, as described above, for the lysimeter samples, except that 1,000 mL of sample water was filtered through the filtering apparatus following the deionized water rinse and before filling the sample bottles.

Lysimeter and piezometer samples were filtered into sample bottles, preserved, and stored on ice until they reached the laboratory for analysis. The DWR Bryte Laboratory analyzed samples for DOC, UVA, THMFP (dose-based), Br, Ca, Mg, K, Cl, SO₄, and alkalinity. Methods of sample preservation and analysis are reported in California Department of Water Resources (1994b). Split samples also were collected for analysis of DOC, UVA, and THMFP by the USGS Laboratory in Boulder, Colo., as described in appendixes B and D. All samples sent to the USGS Laboratory were packed in ice and shipped to be received at the laboratory within 24 hours.

In August 1996, it was determined that other redox parameters should be analyzed, and, in September 1996, the reactivity-based method to determine THMFP was added to the analyses. The redox parameters added to the laboratory analysis included total Fe and Mn and N species (nitrite, nitrate, and ammonium). In addition, a flow-through chamber was used to collect piezometer samples and to monitor other parameters. The reactivity-based method for determination of THMFP is described above in the Trihalomethane Formation Potential section of this report.

Use of the flow-through chamber for monitoring DO, Pt-electrode Eh, and pH allows accurate measurement of these parameters for ground water without contamination by minimizing exposure to atmospheric gases (U.S. Geological Survey, 1980). Water was pumped through the air-tight, flow-through chamber (fitted with DO, Pt-electrode, and pH probes) from the bottom to exclude any air. Meters and probes were calibrated and water was pumped through the flow-through chamber during piezometer development. After at least three casing volumes of water had been pumped through the chamber, DO, Eh, pH, and specific conductance were monitored, and piezometer samples were not collected until these parameters had stabilized (indicated by successive readings not changing by more than about 5 percent). This technique is not applicable to lysimeter sampling because lysimeters usually sample unsaturated soil, and sample degassing and exposure to atmospheric gases affect DO, pH, and Eh measurements. Included in this appendix are tables reporting the DWR Bryte Laboratory

analyses for lysimeter, piezometer, and ditch samples and field parameters. Table A1 lists the field parameters measured (excluding Eh), table A2 presents the mineral data collected, table A3 gives the DOC related analyses and THMFP and haloacetic acid (HAA) results, and table A4 lists the redox-related parameters measured.

APPENDIX B: DISSOLVED ORGANIC CARBON AND ULTRAVIOLET ABSORPTION MEASUREMENTS

Filtered (0.45 mm) samples collected for the measurement of DOC and UVA at 254 nm were packed in ice and shipped to the USGS Laboratory in Boulder, Colo. DOC measurements were made with an OI model 700 total organic carbon analyzer. UVA measurements were made with a Hewlett Packard 8453 spectrophotometer. All measurements were made within 1 week of sample collection. The DOC, UVA, and SUVA data collected during the study are presented in tables B1, B2, and B3 respectively. SUVA is calculated by dividing the UVA values by the DOC concentration. This normalizes the UVA data to carbon and represents the amount of aromaticity per milligram of DOC in a sample.

Quality assurance/quality control (QA/QC) for the DOC and UVA measurements consisted of selecting two or three samples at random and analyzing another sample aliquot (split) at the end of a set of analyses. The QA/QC data and summary statistics for the DOC and UVA measurements are presented in tables B4 and B5 respectively. The difference between a sample and its split can be used to distinguish differences between sample analyses that are significant from those differences caused by random error, which occur in every measurement. For example, if the difference in DOC concentration or UVA between any two samples is less than or equal to the mean differences in tables B4 and B5, then that difference is a result of random error and the samples have essentially the same value. Only if the difference between two samples is greater than the mean difference listed in tables B4 and B5 can we be confident that a significant difference in DOC or UVA values exists.

APPENDIX C: DISSOLVED ORGANIC CARBON FRACTIONATION AND ISOLATION

A major component of this study involved the fractionation (separation) of the DOC into five operationally defined categories and the isolation of the organic matter of two of these categories for analysis by ^{13}C -NMR spectroscopy. These techniques are necessary in the investigation of the role of organic compounds in geochemical processes because the reactivity of an organic compound is determined by its structure, and the DOC content of surface and ground water is composed of thousands of individual compounds, each with its own unique structure. Structure refers to molecular weight and the relative amount of functional groups (carboxylic acid, ether, amine, and so forth). Because each compound has a unique structure and many of these compounds are present in low concentrations, the fractionation and subsequent identification of all compounds individually is impractical. However, it is possible to fractionate compounds with similar structures together into the following operationally defined categories: HPOA, hydrophobic neutrals (HPON), HPIA, hydrophilic neutrals (HPIN), and low molecular weight hydrophilic acids. This fractionation was accomplished with the use of XAD-8 and XAD-4 resin. A description of the physical and chemical properties of these resins is given in Aiken and others (1992).

The HPOA and HPIA fractions constitute the majority of organic carbon in fresh waters. Aiken and others (1992) define HPOA "as that portion of the DOC that sorbs on a column of XAD-8 resin at pH 2 under conditions where k' is 50 for the column, and is eluted at pH 13. This fraction can contain aliphatic carboxylic acids of five to nine carbons, one- and two-ring aromatic carboxylic acids, one- and two-ring phenols, and aquatic humic substances," and they define HPIA "as that portion of the DOC contained in the XAD-8 resin effluent at pH 2 that sorbs on a column of XAD-4 resin under conditions where k' is 50 for the column, and is eluted at pH 13. This fraction can contain polyfunctional organic acids and aliphatic acids with five or fewer C atoms." The capacity factor, k' , is the grams of solute on resin per gram of solute in column void volume.

The ability to isolate compounds that possess similar structures greatly facilitates the study of THM formation. Because the compounds in each operationally defined category have similar structures, each category of organic carbon will react differently with chlorine. For example, Rook (1977) and Reckhow and others (1990) have suggested that the formation of THMs is a result of the reaction of chlorine with aromatic rings in humic substances. Humic substances are classified among the HPOA and are defined as an extremely complex and diverse group of poorly biodegradable decomposition products and byproducts of natural organic matter, which are produced by both plants and animals and whose structure is not well defined (Snoeyink and Jenkins, 1980). Thus, by being able to fractionate the portion of the DOC that is reactive with chlorine, its overall structure can be determined, and the amount of the total DOC that it constitutes can be calculated. This fractionation will lead to a better understanding of the processes controlling the formation and seasonal variability of THM precursors in a given environment and the development of possible land and water use strategies that can be employed to keep the formation of THMs to a minimum.

Samples collected for fractionation and isolation were filtered (0.45 μm) in the field into stainless steel containers, shipped in ice to the USGS Laboratory in Boulder, Colo. and refrigerated until they were fractionated on the columns. The piezometer samples were fractionated on 295-mL capacity columns. These columns process sample volumes of 15 L with a DOC concentration of no greater than 20 mg/L for maximum adsorption efficiency. The piezometer samples always had concentrations greater than 20 mg/L so they were diluted with 0.01 normality (*N*) HCl to a concentration of about 15 to 20 mg/L before being run on the column. The samples were acidified to pH 2 by the addition of 12.1 *N* HCl before they were run on the columns. Acidifying the sample enhances adsorption on the nonionic resin by neutralizing negatively charged species. After acidification, the samples were run on the two XAD resin columns connected in series with Teflon tubing at a constant rate of 60 mL/min. Samples were run through the XAD-8 column first and then through the XAD-4 column. To ensure collection of a sufficient amount of material for analysis by ^{13}C -NMR spectroscopy, enough sample was collected to fractionate each sample through two sets of columns concurrently. After all of the sample was run through the columns, each column was separately back eluted with 0.1 *N* NaOH to desorb the material adsorbed on the column. One liter of 0.1 *N* NaOH was run through the columns at 20 mL/min. and collected in volumetric flasks. The eluates were immediately acidified to pH 2 by the addition of 12.1 *N* HCl.

The next step in the procedure was to remove salts from the samples. After back elution, the eluates were pumped back onto the respective XAD columns and Cl was removed by flushing with deionized water until the water coming out of the bottom of the column had a specific conductance of less than 750 $\mu\text{S}/\text{cm}$. Both columns were again back eluted with 0.1 *N* NaOH and the eluate passed through columns containing hydrogen-saturated cation exchange resin. The cation exchange columns were flushed with deionized water until the specific conductance of the water coming out of the bottom of the column had a specific conductance of less than 50 $\mu\text{S}/\text{cm}$, signifying removal of Na. After the desalting procedure, the sample was freeze dried and analyzed by ^{13}C -NMR spectroscopy.

The lysimeter samples were fractionated on 20-mL columns because a sufficient volume of sample to run them on the 295-mL columns could not be collected, except for a sample collected from lysimeter 2 on January 2, 1997, which was fractionated on the 295-mL column. The 20-mL columns process a sample volume of 1 L with a DOC concentration of no greater than 20 mg/L for maximum adsorption efficiency. The lysimeter samples always had concentrations greater than 20 mg/L, so they were diluted with 0.01 *N* HCl to a concentration of about 15 to 20 mg/L before being run on the column. One liter of sample was run through the XAD-8 column, and the effluent was collected in an Erlenmeyer flask. The XAD-8 effluent was then run through the XAD-4 column and the XAD-4 effluent was collected in an Erlenmeyer flask. The samples were run through both columns at a rate of 4 mL/min. After all the sample was run through the columns, each column was separately back eluted with 100 mL of 0.1 *N* NaOH at a rate of 2 mL/min. The eluates were collected in volumetric flasks and acidified to pH 2 with 12.1 *N* HCl. Because the 20-mL columns process only 1 L of sample, not enough organic material is adsorbed on the resins to isolate for analysis by ^{13}C -NMR

spectroscopy.

The fractionation of the samples onto the XAD-8 and XAD-4 resins allows for a calculation of the mass of each operationally defined category (fraction), expressed as the percent of the original total DOC mass for a particular sample. Data for each fractionation sample collected during the study are listed in table C1. The percent of the total mass that each fraction represents was calculated by summing the masses of each fraction for a sample and dividing the mass of each fraction by the total mass and then multiplying by 100. These fractionation calculations were performed on both runs of the 295-mL columns (table C2).

The mass of each operationally defined category for the 295-mL columns was calculated in the following manner: An initial DOC sample was collected from each sample after it was acidified, but before it was run on the column. Once the sample was running through the columns, a DOC sample was collected from the effluent of each column at 2-L intervals and at the end of the run. Each eluate was analyzed for its DOC concentration before it was desalted. A plot of DOC concentration versus volume of sample passed through the column was made for each sample (fig. C1). The area under the XAD-8 curve represents the mass of C in the XAD-8 effluent. The area under the XAD-4 curve represents the mass of C in the XAD-4 effluent. The total mass of C put on the columns was calculated by multiplying the initial DOC concentration by the sample volume (15 L). The mass of the HPOA fraction was calculated by multiplying the DOC concentration of the XAD-8 eluate by its volume (1 L), and the mass of the HPIA fraction is calculated by multiplying the DOC concentration of the XAD-4 eluate by its volume (1 L). The mass of the HPON fraction is calculated by subtracting the sum of the mass of C in the XAD-8 effluent and the mass of C in the XAD-8 eluate from the initial mass of C put on the column. The HPIN fraction is calculated by subtracting the sum of the mass of C in the XAD-4 effluent and the mass of C in the XAD-4 eluate from the mass of C run through the XAD-4 column (the XAD-8 effluent). The HPON and HPIN are those compounds that adsorb onto the XAD-8 and XAD-4 resins, respectively, but are not dissolved during the back elution with NaOH. The mass of the low molecular weight HPIA fraction is the mass of C in the XAD-4 effluent.

The mass of the operationally defined categories for the 20-mL columns was calculated differently than for the 295-mL columns because the columns were not connected in series. An aliquot of sample, usually 20 mL, was removed from the 1-L sample before being run on the XAD-8 column to measure the DOC concentration of the sample. The sample volume was brought back up to 1 L with deionized water before being run on the XAD-8 column. This procedure was done so that a constant volume was being run through the columns. After the sample was run through the XAD-8 column, an aliquot of the XAD-8 effluent, usually 20 mL, was removed so that the DOC concentration of the XAD-8 effluent could be measured. The volume of the effluent was brought back up to 1 L with deionized water before being run on the XAD-4 column. The DOC concentration of both eluates and the XAD-4 effluent also was measured. The total mass of C put on the columns was calculated by multiplying the DOC concentration of the sample by the sample volume (1 L) minus the volume taken for DOC analysis. The mass of the HPOA fraction was calculated by multiplying the DOC concentration of the XAD-8 eluate by its volume (0.100 L) and the mass of the HPIA fraction was calculated by multiplying the DOC concentration of the XAD-4 eluate by its volume (0.100 L). The mass of the HPON fraction is calculated by subtracting the sum of the mass of C in the XAD-8 effluent (calculated by multiplying the volume of the XAD-8 effluent collected by its concentration) and XAD-8 eluate from the mass of C put on the XAD-8 column. The mass of the HPIN fraction was calculated by subtracting the sum of the mass of C in the XAD-4 effluent (calculated by multiplying the volume of the XAD-4 effluent collected by its concentration) and XAD-4 eluate from the mass of C put on the XAD-4 column. The mass of C put on the XAD-4 column was the product of the concentration of the XAD-8 effluent and the volume of the XAD-8 effluent collected minus the amount taken for DOC analysis. The mass of the low molecular weight HPIA fraction was the mass of C in the XAD-4 effluent.

APPENDIX D: U.S. GEOLOGICAL SURVEY TRIHALOMETHANE METHODS

Background

It has been known for more than 20 years that dissolved natural organic materials (NOM) form THM and other DBPs on chlorination of drinking waters (Rook, 1974; 1977). The formation of DBP during treatment is problematic because of the carcinogenic properties of these compounds (Rook, 1974). However, little is known about the natural processes that form and/or concentrate the chemical precursors to DBP.

The chemical structure of NOM and associated DBP precursors vary with many environmental and chemical parameters, including the source of the organic material, age of the organic material, diagenetic history, soil type, season, molecular size, pH of the source waters, and so forth. Examination of the formation of DBP in waters collected over an annual cycle from different flowpaths in the Twitchell Island study site should provide insights into the sources and processes that lead to elevated NOM concentrations and elevated DBP formation in these waters.

The primary sources of NOM in Twitchell Island pore waters are organic material in the peat soils or degrading above- and below-ground crop biomass. Secondary heterotrophic productivity may alter the chemical composition of the organic material and thus either concentrate or degrade DBP precursors. Some NOM (only 2 mg/L as DOC) is present in water siphoned onto the island, but represents only a small fraction of NOM present in the samples analyzed as part of this study.

Given the sources, NOM can arise from three distinct soil zones: the upper oxidized zone, the lower reducing zone, and the zone periodically exposed to oxidizing and reducing conditions during water-table fluctuations. The extent of each of these zones can vary seasonally, depending on irrigation practice, intentional winter flooding, and seasonal rainfall.

As discussed in the body of this report, samples were collected from two soil zones for determination of THMFP and pertinent physical and chemical parameters. Ancillary physical and chemical parameters are reported in appendix A.

The Chemistry of Disinfection Byproduct Formation

Dissolved chlorine, used for disinfection of drinking water, disproportionates to hypochlorous acid (HOCl) and chlorine. HOCl, in turn, rapidly reacts with ammonia to form chloramines and with Br to form hypobromous acid (HOBr). Together, HOCl, HOBr, and chloramines react with inorganic and organic compounds present in the treated waters. In particular, they react with NOM to form THM and other DBP.

The reactions that form THM are additions and substitutions to chemical structures commonly found in NOM, generally additions to olefins and substitutions in aliphatic and aromatic compounds. The abundance of these chemical structures determines the speed and extent to which THM forms on treatment, as well as they carry information about the source and diagenetic history of the NOM (Reckhow and Edzwald, 1989).

Thus, intrinsic, source-related properties of the reactive chemical structures in NOM determine the amount of THM formed during chlorination. Several studies have been undertaken with model compounds to provide insights into the reactivity of structures within NOM (Rook, 1976; 1977). Aromatic structures, such as substituted benzenes, and other electron-rich structures, such as ketones, generally are reactive, but reactivity estimates for a variety of olefinic structures were unavailable. Reactions generally can be grouped into the aromatic type and ketone type for the purpose of understanding the activity of NOM structures.

In addition to the intrinsic chemical structure of the NOM, the speed and extent of formation of THM

during chlorination depend on a number of physical and chemical parameters. The chlorine dose, the duration of the reaction, the presence of bromide and ammonium, the NOM concentration, and the temperature and pH at which the reaction is conducted contribute to the amount of THM formed. Therefore, controlling these parameters when comparing differences in THMFP among samples and making sure all samples were analyzed using comparable tests are important.

Methods Employed by the U.S. Geological Survey Laboratory for Trihalomethane Formation Potential Analysis

Several tests for the capacity of natural waters to form DBP are currently in use (Reckhow and Edzwald, 1989). The results obtained using these tests are highly dependent on the method used, so care should be taken in comparing data using different analytical methodologies. Two different tests were conducted as part of this study to determine the THMFP of the tested waters. The majority of the data was generated using the dose-based test (THMFP_{db}), in which a fixed amount of chlorine is added to the sample (California Department of Water Resources, 1994b). Data of this type were collected throughout the study and were used to generate the seasonal comparisons. The second type of test was the reactivity-based test (THMFP_{rb}), in which the chlorine demand is calculated to determine chlorine dose. For an accounting of which THMFP method was applied to which samples and how the results of different methods compare, see the Trihalomethane Formation Potential section. The THMFP tests used here are significantly different from the commonly used simulated distribution system (SDS) test.

Whole-water samples were kept refrigerated until analyzed and were analyzed within 1 week of collection. The freeze-dried eluates were prepared for the THMFP test by dissolving 5 mg of material in 0.5 L of a 0.001N NaHCO_3 solution. Aliquots of the sample were taken for DOC and UVA measurements. The rest of the sample was then treated in exactly the same manner as the whole-water samples.

To determine the chlorine demand of each sample, prior to chlorination, the DOC and ammonia concentrations were determined for all samples. All samples were chlorinated with a dose based on the inorganic and organic demand according to the following formula, based on the method of Krasner and Scilimenti (1993):

$$[\text{Cl}_2] = (3 \times [\text{DOC}]) + (7.6 \times [\text{NH}_3])$$

Ammonia concentrations were determined using a HACH kit according to the method 8038 of the HACH Water Analysis Book (HACH, 1992). The procedure employs direct nesslerization of whole-water samples, reacting 25 mL of each sample with Nessler reagent for 1 minute, then measurement of solution color against a blank on a spectrophotometer at 425 nm. DOC measurements were made in duplicate by persulfate oxidation on a Oceanics International model 700 TOC analyzer.

The THMFP_{rb} for each sample was determined using a modified version of method 5710 B described in Eaton (1995). Briefly, samples were buffered to pH 8.4 with a freshly prepared $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}/\text{Na}_2\text{HPO}_4$ buffer solution, and the pH corrected to 7 using 0.5 M HCl or 0.5 M NaOH. Samples were then transferred into a 72-mL serum vial and sealed using a Teflon-faced septa without leaving a headspace (to prevent volatilization of THM). The chlorination solution was injected by syringe through the septum, allowing the displaced volume to exit through a second syringe. The bottles were then incubated for 7 days at 20°C in a controlled-temperature recirculating bath.

The chlorination reaction was terminated by pouring 30 mL of the sample into a 40-mL screw-top vial fitted with a Teflon-faced septa, followed by 2 mL of sodium sulfite solution (1.5 M). Chlorine residual was determined on the remaining sample using HACH procedure 8167 (HACH, 1992).

Samples were extracted from the aqueous phase by first adding sodium chloride (8 g) to reduce solubility of ether and to increase partitioning of THM into the organic phase, vigorously shaking the vial for 20 seconds, then adding 3 mL of methyl tert butyl ether and shaking for 2 minutes. After allowing 10 minutes for separation of the organic and aqueous layers, 1.5 mL of the organic layer was removed to an autosampler vial using a Pasteur pipet and then immediately sealing the vial.

Quantification of THM was accomplished using a Hewlett Packard 5890 II gas chromatograph fitted with an auto injector, a capillary split-splitless inlet, and a Nickel⁶³ electron capture detector. Baseline chromatographic separation was obtained using a DB-1 fused silica capillary chromatography column (30 M x 0.25 mm i.d.). The column oven was programmed to hold an initial temperature of 40°C for 4 minutes, increased to a final temperature of 100°C at a rate of 15°C/min, and held for 3 minutes.

THMs were identified by comparing the peak retention time to that of authentic standards. THMs were quantified by comparing the peak area of sample analyses to the areas resulting from the injection of calibration standards of known concentration. THMFP data obtained using this method for samples from this study are presented in [table D1](#).

APPENDIX E: CARBON-13 NUCLEAR MAGNETIC RESONANCE ANALYSES

Introduction

The study of the nature, reactivity, and environmental significance of organic matter in aquatic systems is hampered by the inherent chemical complexity of the organic matter. Fractionation of DOC on XAD resins results in fractions of the DOC that are themselves complicated, heterogeneous mixtures. Typically, the complicated mixtures obtained from the fractionation of DOC into compound classes are characterized by elemental, molecular weight, acid-base titration and amino acid analyses, and by ¹³C-NMR, ¹H-NMR, and IR spectroscopy. The fact that the fractions are complex, heterogeneous mixtures limits the amount of information that can be obtained about the composition of the mixture using each of the above methods, but these techniques do provide valuable structural and functional group information that, taken as a whole, can be used to establish the nature and source of organic isolates.

Selected samples collected during this study were analyzed by ¹³C-NMR to learn more about the differences in chemical composition between different sampling locations and to relate the reactivity of these isolates to their chemical composition. ¹³C-NMR is an important technique for obtaining structural information on complicated mixtures of organic compounds, such as humic substances and coal. In recent years, use of this technique to study humic substances has increased dramatically, and a number of review articles describing the technique and its application to environmental studies have been published (Wershaw, 1985; Wershaw and Mikita, 1987; Preston, 1987; Steelink and others, 1990; Cook and others, 1996).

Two different ¹³C-NMR techniques were applied in this study. Quantitative spectra were obtained on two samples using liquid state ¹³C-NMR. This technique is time consuming and requires a relatively large amount of sample. Two samples were analyzed by liquid state NMR. A larger set of samples was also analyzed by cross-polarization (CP) magic angle spinning (MAS) ¹³C-NMR analysis, which provides qualitative data that allow comparison between samples. This method has the advantages of short analysis times and small amounts of sample. Unfortunately, there are problems inherent to this technique that interfere with the acquisition of quantitative spectra (Cook and others, 1996).

In all cases, the ¹³C-NMR spectra are comprised of six major bands. The general assignments for

these major bands are as follows:

1. Aliphatic I (0-60 ppm)--primarily sp³ hybridized carbons bonded to other carbons; carbons bonded to nitrogen and sulfur also can occur in this region. Methoxyl carbons occur in the approximate range from 54 to 60 ppm;
2. Aliphatic II (60-90 ppm)--hetero-aliphatic carbons, primarily sp³ hybridized carbons bonded to oxygens, including ether, alcohol, and carbohydrate carbons;
3. Acetal (90-110 ppm)--acetal and ketal carbons and anomeric carbons of carbohydrates;
4. Aromatic (110-160 ppm)--primarily aromatic and olefinic carbons;
5. Carboxyl (160-190 ppm)--carboxylic acid carbons. Ester, amide, and lactone carbons also can overlap with the carboxyl carbons; and
6. Ketone (190-230 ppm)--primarily ketones and aldehydes.

In general, the broad-banded nature of the spectra presented in this report indicates that the samples are complex organic mixtures.

Results

Quantitative ¹³C-NMR spectra for the HPOA fractions collected from the drainage ditch (March 1996) and Piezometer 3 (March 1996) are presented in figures E1 and E2. In general, these spectra are very similar. As discussed in the Description of Study Site and Study Design section of the report, there appeared to be a connection between the drainage ditch and site 3, and this similarity further confirms this hypothesis. The most prominent peak in each spectrum is the carboxyl peak, which indicates that these fractions of the DOC are organic acids. Each sample also has a large response in the aromatic carbon, which indicates that the samples are highly aromatic in nature. Integrating the spectra provides the percent distribution of carbon types in the samples. These data are presented in table E1. Compared to similar samples from other environments (fig. E3), the samples from Twitchell Island are more aromatic and contain fewer aliphatic-II and anomeric carbon atoms associated with carbohydrates. The differences associated with these samples are due, in large part, to differences in sources of dissolved organic matter in each environment. Based on a large data set, the Lake Fryxell and Suwannee HPOA samples represent extremes with regard to aromatic carbon content. The Lake Fryxell sample represents organic matter derived from algae and bacteria, whereas the Suwannee sample is derived largely from lignin-containing plants. The Twitchell Island samples are more aromatic than even the Suwannee sample. The large amount of aromatic carbon associated with the Twitchell Island samples is of significance in this study because of the enhanced reactivity of aromatic molecules with chlorine leading to the formation of DBP.

Intercomparison between the samples collected on Twitchell Island can be done by comparing data obtained from CPMAS ¹³C-NMR analyses. Although these data are not quantitative, qualitative differences between the samples can be identified. Representative spectra are presented in figures E4 and E5, whereas integration data are presented in table E2. Note that the ketone region (190-230 ppm) has not been included in this table because of the presence of spinning side bands in this part of the CPMAS spectrum. In addition, the integration values in the aliphatic region of the CPMAS spectra are a much greater percent, whereas the aromatic and carboxyl regions are a much lower percent relative to the data discussed above for the quantitative liquid state measurements. These differences in the spectra result from problems inherent in the CPMAS technique that are beyond the scope of this report. Interested readers are referred to the paper by Cook and others (1996) for a more detailed discussion of the difficulties in obtaining quantitative information by CPMAS ¹³C-NMR. This technique is useful, however, for an intercomparison of samples from the same location.

Differences between the HPOA and HPIA fractions are evident from a comparison of the spectra obtained for samples from piezometer 4 (figs. E4 and E5). The HPOA fraction has a more prominent peak in the aromatic carbon region (110-160 ppm) and a less prominent peak in the aliphatic-II region (60-90 ppm). The integration data in table E2 indicate that, for each sample, the HPIA fractions have greater carboxyl, aliphatic-II, and aliphatic-I carbon contents and are less aromatic than the corresponding HPOA fractions. These differences are apparent in the bar graph comparison of the NMR data presented for piezometer 4 in figure E6. From the ^{13}C -NMR data, the major structural differences between the two fractions are that the HPIA fraction is less aromatic than the fulvic acid and has a greater amount of carboxyl and heteroaliphatic carbon. On the basis of the greater aromatic carbon content of the HPOA fractions, this fraction of the DOC is expected to be more of a factor with regard to the generation of DBP.

A comparison of the differences between different sites for a given sampling date (figs. E7 and E8) shows that there are few differences in the composition of either the HPOA or HPIA fractions. The similarities in composition for the isolates from the piezometer samples reflect similarities in the source materials, for example, the peat soils, and in the processes leading to the generation of DOC from these soils. These similarities suggest that dissolved organic matter of similar composition and reactivity would be obtained from other fields that have similar soils and land use. The compositional similarity of the samples collected from the drainage ditch and at the pump location to those samples obtained from the piezometers confirms one of the original hypotheses of this study, that the nature of the soil organic matter exerts a strong influence on the chemical composition and reactivity of the organic matter transported off the island.

Finally, samples of the peat soils also were characterized by CPMAS ^{13}C -NMR (fig. E9). In general, the soil samples were observed to be more aliphatic and to contain greater amounts of carbohydrates, as evidenced by the aliphatic-II peak compared to the HPOA fractions from the lysimeters and the piezometers. The soil samples also contained significantly less carboxyl content than the dissolved HPOA fractions. These results suggest that the aromatic organic acids are removed from the peat at a faster rate than other components of the peat and that the aliphatic and carbohydrate (probably cellulose) rich fractions of the peat organic matter are selectively preserved.

It is interesting to compare the spectra for soil samples collected from depths in the soil profile that have different redox conditions. The lysimeters were 6 to 18 in. below the surface. At this depth, the soils are exposed to oxygen and are classified as oxidized. The piezometers, on the other hand, were below the water table at a depth of 54 to 72 in. in a reduced zone with little oxygen. Exposure of the peat soils to oxygen results in accelerated rates of peat oxidation, a mechanism that is largely responsible for the subsidence of soils on the peat islands as a result of wetland drainage and subsequent cultivation. CPMAS ^{13}C -NMR spectra for soil samples collected near lysimeters 2 and 4 are compared with those from piezometers 2 and 4 in figures E9 and E10. As expected, the shallow samples are similar to each other, as are the deeper samples. Differences between the shallow and deep soils can be attributed to enhanced degradation of the shallow soils. The lysimeter samples are less aromatic and have less aliphatic II carbon associated with carbohydrates than the piezometer samples. The shallow samples also are enriched in carboxyl content and aliphatic I carbon. These results are consistent with greater degradation rates for the aromatic, and to a lesser extent, carbohydrate moieties in the oxidized soils compared to the reduced soils. Carboxyl groups are a product of microbial oxidation and also are consistent with a greater degree of oxidation of the shallow soils.

More detailed examination of the aromatic region of the NMR spectra for the soils indicates that the region of the spectra associated with aromatic phenols (140-160 ppm), such as tannins and lignin phenols, is depleted at a faster rate than the aromatic moieties that do not have hydroxyls associated with them (110-140 ppm). Associated with the loss of aromatic phenols is the loss at about 57 ppm of what is likely to be methoxy ethers. A model for degradation that would account for this difference involves the oxidation of aromatic phenols with the subsequent opening of the aromatic ring,